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September 7, 1973

Dear Dr. Nathans,

I have followed with interest yout analysis of SV-40. At present my laboratory is using the <u>Hin</u> restrictase to carry out a similar study on T7 so perhaps we might usefully establish contact. This should help to standardise nomenclature, techniques etc. in what is obviously developing into an enormous field. At present I doubt that I have much information which will be of interest to you but I will tell you the state of our analysis anaway in case there is something useful, and I have one or two requests.

But, first of all, I have a comment on your nomenclature which arises from our own experience with T7 DNA (mol.wt. 23x106, 40,000 base pairs). We obtain about 50 fragments from T7 DNA after Hin digestion, so alphabetical nomenclature becomes impossible or clumsy. We have adopted a numerical nomenclature for bands on gels Hin 1, Hin 2 etc. which conflicts with your use of this nomenclature for cleavage sites. As larger DNA molecules are a priori likely to be cleaved into larger numbers of fragments, it seems more reasonable that the whole system should be numerical. The confusion with site nomenclature then would be solved if Roman numerals were used for sites and Arabic numerals for bands and this is the system we have decided on for T7.

Because of the large number of fragments from T_7 some are not separated on gels and so our nomenclature refers specifically to bands on the gels. So Hin 12 from T_7 is a band which in fact probably contains 3 fragments of similar size, not resolved by the gel - this we indicate as Hin 12a-c. It is possible that these fragments may be separable in the future when they would be designated Hin 12a, Hin 12b and Hin 12c.

This 'nomenclature bit' is tedious but that is our story on it - would be grateful for comments.

Our findings on T7 so far with Hin digestion are in summary as follows:

- 1. 41 bands identified on 40 cm 3% polyacrylamide gels.
- 2. Have used ³²P DNA, ³H DNA and methylene blue staining to analyse R_F and DNA conc. per band.
- 3. We concluded that some bands contained 2 fragments per band while band 12 contained 3.

- 4. We are interested in the early region of T₇. We have isolated deletions which we think affect this region which stretches 1-20% of the T₇ molecule. <u>Hin</u> digest of deletion PC 1, differs from wild type in that <u>Hin 8</u>, <u>Hin 28</u> and <u>Hin 30</u> disappear and there is a new band between <u>Hin 13</u> and <u>Hin 14</u>.
- 5. We are at present learning E.M. to analyse the map position of all fragments.
- 6. I enclose a preprint of a paper submitted to Virology which contains some of this data.
- 7. You will see that we have calculated that the T₇ Hin fragments vary in size from about 1200 bases to 200 bases. I can get a linear plot which I would expect from Fig. 2. Danna et al., J.M.B.78, 363(1973), since I calculate that T7 Hin 1 is about the same size as SV40 Hin A and T7 Hin 41 is about the same as SV40 Hin K. You could plot Nin A Hin K as a straight line:

I am anxious to calculate the size of the T₇ <u>Hin</u> fragments more accurately but I need standards to do this. I would be very grateful if you could send me 2x10^o cpm of a <u>Hin</u> digest of ³²P SV40 (air mail please!) which we would use as standard. Unfortunately we have no tissue culture facilities so we cannot make our own and I have no idea whether our request is simple from your point of view.

In return is you needed it sometime we would be delighted to send you $\underline{\text{Hin}}$ T7 DNA fragments either 3H or 32P.

We have heard rumours, and the <u>J.M.B.</u> paper confirms them that <u>H. influenzae</u> has at least 3 endonucleases, one which requires SAM and ATP (Gromkova, <u>J. Bact.</u>) and two which do not. Can you tell us how these last two are separated from each other?

I would also like to say that any time you are flying over Ireland on the way to or from Europe, we would be delighted if you would break your journey in Dublin, giving us a seminar. We would of course carry your expenses in Dublin and there would be a very small honorarium, but unfortunately that is about all we could do. I think you would enjoy the experience - it is a pleasant city to visit.

Looking forward to hearing from you.

Yours sincerely.

Dr. David McConnell

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